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EXAMINER	
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ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/607,903	HUISMAN ET AL.	
	Examiner	Art Unit	
	Richard G. Hutson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 September 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23* is/are pending in the application.

4a) Of the above claim(s) *11-23* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment of claims 1, 9, 11, in the paper of 9/22/2006, is acknowledged. Claims 1-23 are still at issue and are present for examination.

Applicants' arguments filed on 9/22/2006, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claims 11-23 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-10 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

This rejection was stated in the previous office action as it applied to previous claims 1-10. In response to this rejection applicants amended claims 1 and 9 and traverse the rejection as it applies to the newly amended claims.

Applicants submit that the inventor had possession of the claimed invention at the time of filing. Applicants submit that genetic engineering of bacteria has been practiced for decades and that commercial fermentation of bacteria to produce amino acids and other products as well as the mutagenesis of bacteria and screening for a particular characteristic such as antibiotic resistance, or increased production or tolerance to a product such as alcohol is totally routine. Thus all that is required is that one expose the bacteria to a known mutagen, then subject the bacteria to a screen for whatever is the desired product, such as an increase in the production and secretion of a nuclease. Applicants further submit that bacterial strains such as *Ralstonia, Aeromonas, Azotobacter, Burkholderia, Comamonas, Methylobacterium, Paracoccus, Pseudomonas, Rhisobium,* and *Zooglea* have been sold by the American Type Culture Collection ™ in Rockville MD and suitable nuclease genes were well known and described in the literature as well as the means of isolating these genes, as of the date of filing of this application.

While knowledge of one skilled in the art is relevant to meeting the written description requirement, applicant is reminded that the instant rejection is based on a lack of written description, not a lack of enablement. Regardless, applicants argument is not persuasive because while bacterial strains and nuclease genes for use in the making of the claimed mutants, as well as methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc., are well known to the skilled artisan, producing variants as claimed by Applicants (i.e., a bacterial strain comprising any heterologous nuclease gene as well as any genetic modification of any homologous nuclease gene such that expression or modification is an amount effective

to degrade nucleic acid so that recovery of a product is enhanced) requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the claimed property. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute undue experimentation. While the statute is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification as the specification discloses only the species of the claimed genus encompassed by *P. putida*, *R. eutropha* and *E. coli*, expressing the heterologous *Staphylococcus aureus* nuclease gene, *nuc*, which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus.

The genus of bacterial strains that are claimed is a large variable genus comprising any bacterial strain comprising any heterologous nuclease gene as well as any genetic modification of any homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. While one of skill in the art may have used routine experimentation to laborisly arrive at those strains encompassed by the instant claims which comprise a genetic modification of a homologous nuclease gene, such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced, applicants were clearly not in possession of such strains, as applicants do

not describe a single bacterial strain which comprises a single genetic modification of a homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Applicants submit that the application contains examples demonstrating that one can genetically engineer known strains of bacteria with known nuclease genes to make a product as claimed, however as discussed above this rejection is not based on a lack of enablement, but rather a lack of written description, and as discussed above, applicant is reminded that applicant examples merely teach the isolation of the *Staphylococcus aureus* nuclease gene *nuc* and the integration of this isolated heterologous nuclease gene into three different bacterial strains, *P. putida*, *R. eutropha* and *E. coli*. Applicants have not taught a single bacterial strain comprising a genetic modification of a homologous nuclease gene such that expression or its modification results in an amount effective to degrade nucleic acid so that recovery of a product is enhanced.

As stated previously and above, the specification fails to describe representative species of these bacterial strains by any identifying structural characteristics or properties other than the functional characteristics recited in the claims, for which no predictability of structure is apparent. There is no disclosure of any particular structure to function/activity relationship in the disclosed species with respect to those heterologous nuclease genes or those genetic modifications of homologous

nuclease genes such that expression or modification is in an amount effective to degrade nucleic acid so that recovery of a product is enhanced.

The genus of bacterial strains that are claimed is a large variable genus comprising any bacterial strain comprising any heterologous nuclease gene as well as any genetic modification of any homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. The specification discloses only the species of the claimed genus encompassed by *P. putida*, *R.. eutropha* and *E. coli*, expressing the heterologous *Staphylococcus aureus* nuclease gene, *nuc*, which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus. Applicants do not describe a single bacterial strain which comprises a genetic modification of a homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Applicants comments with respect to the rationale for the inclusion of claims 2-5 and 7, drawn to those bacterial strains capable of growth to cell densities of at least 50g/l (claim 2), those bacterial strains which produce a polyhydroxyalkanoate to a level of at least 40% of its dry weight (claim 3), those bacterial strains for use in an aqueous process to manufacture poly(3-hydroxyalkanoate) which is essentially free of nucleic acids (claim 4), those bacterial strains for use in a process for making any of a number of different polysaccharides (claim 5), or those bacterial strains selected from the group

consisting of *Ralstonia eutropha, Methylobacterium organophilum, Methylobacterium extorquens, Aeromonas caviae, Azotobacter, vinelandii, Alcaligenes latus, Pseudomonas oleovorans, Pseudomonas fluorescens, Pseudomonas putidas, Pseudomonas aeruginosa, Pseudomonas acidophila, Pseudomonas resinovorans, Escherchia coli, and Klebsiella* (claim 7) are acknowledged. Claims 1-5 and 7 as well as the additional dependent claims 8-10 are included in this rejection for the same rationale that independent claim 1 has been rejected above, as none of these dependent claims sufficiently limit independent claim 1 such that the claimed genus was considered to be adequately described. Each of the dependent claims are drawn to those bacterial strain comprising any heterologous nuclease gene as well as any genetic modification of any homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. As discussed above, applicants do not describe a single bacterial strain which comprises a single genetic modification of a homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 4, 5, 6 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Liebl et al. (J. Bacteriology 174(6): 1854-1861 (1992)).

This rejection was stated in the previous office action as it applied to previous claims 1, 2, 4, 5, 6 and 8. In response to this rejection applicants amended claims 1, 9 and 11 and traverse the rejection as it applies to the newly amended claims.

As previously stated, Liebl et al. teach the expression, secretion and processing of *Staphylococcal aureus* nuclease by *Corynebacterium glutamicum*. Liebl et al. teach that Coryne*bacterium glutamicum* is closely related to other "amino acid-producing corynebacteria" and these organisms are used for the industrial production of certain amino acids. Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease. Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme.

Therefore, Liebl et al. anticipates claims 1, 2, 4, 5 and 8 drawn to a bacterial strain wherein the bacterial strain expresses a heterologous nuclease gene. It is noted that with respect to claims 4 and 5, that Liebl et al. do not teach that this bacterial strain is used in a process to manufacture poly(3-hydroxyalkonates) or polysaccharides, but

since these are uses of the recited bacterial strain these are not patentable limitations of the claimed bacterial strain, thus these claims are included in the rejection. While Liebl et al. do not teach the limitations of claims 2 and 8 with respect to density of growth and amount of enzyme produced, respectively, it is believed that these are inherent properties of the disclosed bacterial strain absent some teaching to contrary. As shown in Figure 2, Liebl et al. show that one of the taught bacterial strains, *C. glutamicum* R163/pWLQN10, produced approximately 80-fold higher SNase activity after induction as a result of IPTG supplementation of the medium and this strain grew approximate density of OD_{600} = 5.0 (Figure 2 and supporting text at page1859, right column, last paragraph before Discussion). Compare this optical density to that of the instantly disclosed strains MBX978 and MBX 985 which Applicants disclose as having an OD_{600} of 3.7 and 3.8 at the end of disclosed experiments (Table 1 of instant application).

Applicants traverse the rejection on the following basis. After summarizing the legal standard and applicant's interpretation of the claims, applicants submit their interpretation of what the reference Liebel et al teaches. Applicants submit that Liebel et al. does not teach "the reduction of viscosity of a cell lysate as a direct result of secreting a nuclease" or a bacterial strain for the production of a fermentation product. Applicants further submit that the vast majority of bacterial strains will not produce a nuclease in an effective amount to reduce the viscosity to facilitate product recovery. Applicants submit that Liebel et al. does not screen for strains for production of a product.

Applicants further submit, with respect to claims 2 and 4, that there is no teaching in Liebl of a fermenting bacterial strain capable of growing to densities of at least 50g/L and with respect to claim 5, there is no teaching in Liebl of a process for making polysaccharides.

Applicants submit, with respect to claim 8, there is no teaching in Liebl to suggest an amount of nuclease that is present to degrade at least 95% of all nucleic acid released following lysis of the cells in less than 24 hours.

Applicant's further support applicant's position, by submitting examples of applicant's accomplishments as disclosed in the specifications' examples. Applicants thus conclude that based upon what applicants submit Liebl does and does not teach the reference of Liebl cannot anticipate claims 1, 2, 4, 5, 6 and 8.

Applicant's complete argument is acknowledged and has been carefully considered, however, is not found persuasive on the following basis. While applicants submission of what Liebl et al. does not teach is appreciated, the rejection is based upon what Liebl et al. does teach and many of the "teachings" applicants refer to are irrelevant to the anticipation of the claimed invention.

Applicants traverse this rejection on the basis that while Liebl et al. teaches *Staphylococcal* nuclease (SNase) expression by various *C. glutamicum* strains, applicants point out that Liebl et al. does not teach the enhancement of product recovery as a direct result of secreting a nuclease into the periplasm or growth medium and there is no showing of an effective amount of secreted nuclease activity to enhance product recovery. Such a teaching that the enhancement of product recovery is a direct

result of secreting a nuclease into the periplasm or growth medium by the taught bacterial strain is not a limitation of the anticipated claims, however as Liebl et al. teach the strain R163/pWLQN10, which produced approximately 80-fold higher SNase activity after induction as a result of IPTG supplementation of the medium, it is believed that this increase in the production would result in a reduction of the viscosity of the medium such that product recovery would be enhanced, even if only a by a small amount.

Applicants comments that Liebl et al. does not screen for nor identify any strains having the desired characteristics is acknowledged, however, as stated above, Liebl et al. teach and produce a bacterial strain which anticipates the claimed bacterial strains.

Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease gene at a high level and excrete the *Staphylococcal aureus* nuclease (See page 1856, bottom of right column, *Overexpression of SNase in C. glutamicum*). Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme. The taught bacterial strain is capable of producing amino acids, polyhydroxyalkonates or polysaccharides and the secretion of the nuclease into the growth medium by the taught bacterial strain would result in an effective amount of secreted nuclease activity to degrade nucleic acid so that recovery of a product is enhanced. As discussed above, the taught bacterial strain of Liebl et al. is capable of growth to cell densities of at least 50g/l. This is supported by the teachings of Liebl et al., in which they show that the taught bacterial strains, *C. glutamicum* R163/pWLQN10, grew to an optical density of

approximate OD_{600} = 5.0 (Figure 2 and supporting text at page1859, right column, last paragraph before Discussion). Compare this optical density to that of the instantly disclosed strains MBX978 and MBX 985 which Applicants disclose as having an OD_{600} of 3.7 and 3.8 at the end of disclosed experiments (Table 1 of instant application). Further, the bacterial strain of Liebl et al., *C. glutamicum* R163/pWLQN10 inherently expresses the nuclease in an amount effective to degrade at least 95% of all nucleic acid released following lysis of the cells in less then 24 hours. This assessment of the level of expression of the nuclease expressed by the bacterial strain taught by Liebl et al. is based on the level of nuclease expressed by the *C. glutamicum* strain R163/pWLQN10, which produced approximately 80-fold higher SNase activity (approximately 40 Units /ml after 24 hours of culture) after induction as a result of IPTG supplementation of the medium, and the fact that the *staphylococcal* nuclease is heat-stable and would thus be an amount effective to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. It is acknowledged that an amount effective to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours is a somewhat broad limitation as a single cleavage event of a nucleic acid molecule could be considered to be encompassed by "degrade"., and thus the bacterial strain taught by Liebl et al. secretes the expressed nuclease in an amount effective to degrade at least 95% of all nucleic acid released following lysis of the cells in less then 24 hours.

Further, the bacterial strains taught by Liebl et al. are capable of being used for an aqueous process to manufacture poly (3-hydroxyalkanoates) (claim 4) or capable of

being used in a process for making selected polysaccharides (claim 5), in spite of Liebl et al. not teachings such processes. Applicants are reminded that the these intended uses of the claimed bacterial strains have no patentable weight as they do not further limit the claimed bacterial strains of claims 2 and 1.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 1-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

This rejection was stated in the previous office action as it applied to previous claims 1-10. In response to this rejection applicants amended claims 1 and 9 and traverse the rejection as it applies to the newly amended claims.

As previously stated, Greer et al. teach that the degradation or removal of nucleic acids from cell lysates during fermentation is important because they form solutions of

high viscosity which interfere with subsequent processing. Greer et al. specifically teach the usefulness of peroxide degradation in the recovery of intracellularly produced materials, in particular polyhydroxyalkanoate polymers, from bacterial cell lysates. Greer et al. further teach that nucleases can also be added to a cell lysate in order to degrade the nucleic acid although nucleases are expensive (page 1, lines 25-31).

Liebl et al. teach the expression, secretion and processing of *Staphylococcal aureus* nuclease by *Corynebacterium glutamicum*. Liebl et al. teach that Corynebacterium glutamicum is closely related to other "amino acid-producing corynebacteria" and these organisms are used for the industrial production of certain amino acids. Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease. Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme.

Miller et al. teach the secretion and processing of *Staphylococcal aureus* nuclease in *Bacillus subtilis*.

Atkinson et al. teach all aspects of biochemical engineering and biotechnology, including properties of microorganisms, microbial activity, product formation, fermentation processes, downstream processes and product recovery processes. Atkinson et al. also teach many products that can be produced biochemically such as antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides. Atkinson et al. specifically teach many of the industrial production characteristics for a number of commercially important compounds, for example

Atkinson et al. teach that Alcaligenes eutrophus has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32).

Lee et al. teach several processes developed for the production of various poly(hydroxyalkanoic acids) including various microorganisms used and the optimization of fermentation conditions.

One of ordinary skill in the art would have been motivated to genetically engineer a bacterial strain to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of a fermentation process for the synthesis of industrially important molecules. A nuclease excreted into the medium as a result of such a genetically engineered bacterial strain would a inherently result in the degradation of at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would

not require an external nuclease or hydrogen peroxide to be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium. Alternatively one would have been motivated to engineer a homologous nuclease to increase its nuclease activity for the same reasons as stated above for the introduction of the heterologous *Staphylococcal* nuclease.

Further, one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. in order to more efficiently produce the desired product, consisting of antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides as taught by Atkinson et al. Optimization of fermentation conditions includes the choice of the bacterial host such as *Methylobacterium organophilum*, *Methylobacterium extorquens*, *Alcaligenes eutrophus*, *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Pseudomonas resinovorans*, *Pseudomonas acidovorans* and *Escherichia coli* or any other microorganism which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that Alcaligenes eutrophus has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell

densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32). It would have been obvious to use a bacterial strain which grows to a high cell density and/or which produces a high level of the desired product.

Applicants traverse this rejection on a number of different basis. In response to applicants arguments, many of which are made against the references individually, Applicant is reminded that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

First, applicants submit as above, that Leibel et al. does not teach possible uses for the disclosed nuclease other than for elucidating protein export and processing mechanisms. Applicants comments regarding Liebl et al. are not persuasive because any use that may or may not be taught by Liebl et al. is not important in the instant rejection because the intended use of the claimed bacterial strain does not limit the claimed bacterial strain and further because Liebl et al. is not used to show motivation for the creation of the claimed bacterial strain, but rather Liebl et al. is relied upon to show that there is a reasonable expectation of success of producing the claimed bacterial strain. Greer et al. is relied upon for the motivation to create the claimed bacterial strains because as discussed above, the motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the

viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme.

Applicants further argue that "any amount of nuclease excreted into the medium would not necessarily result in a decrease in the viscosity of the medium and therefore an enhancement of product recovery. Applicants state that this is demonstrated by the examples, in which applicants submit that the levels of expression are compared in Table 1 of the instant specification. Applicants comments support that different levels of expression for different bacterial transformants can be expected, however applicants point is unclear as applicants have merely labeled the relative nuclease activity of the different strains by a number of "+" marks, and not shown any data as to how the "different levels of nuclease expression" correlates with the "effectiveness to degrade nucleic acid" so that recovery of the product is enhanced.

Finally, Applicants comments with respect to the reference Liebl et al. and what is an "effective amount of nuclease to decrease the viscosity of the medium" are somewhat unclear as this is a obviousness type of rejection and Applicants appear to argue that one of ordinary skill in the art of protein/product purification from microorganisms will realize that an effective amount does not correlate with just "any amount of nuclease excreted into the medium". This in combination with Applicants earlier comments above, under the above 112 first paragraph rejection, based on a lack of written description, in which Applicants submitted that genetic engineering of bacteria has been practiced for decades and that commercial fermentation of bacteria to produce amino acids and other products, as well as the mutagenesis of bacteria and screening for a particular characteristic such as antibiotic resistance, or an increase in the production and secretion of a nuclease, would seem to support the rejection that determination of and engineering of "an effective amount of nuclease to degrade nucleic acid so that recovery of the product is enhanced" is desirable and well within the ability of the ordinary artisan.

As with the reference Liebl et al. above, Applicants submit that there is no teaching in Miller et al. to select for strains which secrete into the periplasm or growth medium so that recovery of a large scale product is enhanced. As above these arguments are not persuasive because any use or means of making the taught bacterial strain, that may or may not be taught by Miller et al. is not important in the instant rejection because the any use or means of making the taught bacterial strain does not limit the bacterial strain and further because Miller et al. is not used to show motivation

for the creation of the claimed bacterial strain, but rather Miller et al. is relied upon to show that there is a reasonable expectation of success of producing the claimed bacterial strain. Greer et al. is relied upon for the motivation to create the claimed bacterial strains because as discussed above, the motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme.

Applicants point out that one of ordinary skill in the art will realize that a nuclease that is only secreted into the periplasm of a bacterial cell must be released in order to exhibit the necessary extracellular activity to degrade nucleic acid so that recovery of the product is enhanced. While this is acknowledged, Applicants are reminded that the

rejected claims currently recite "and secrete the nuclease into the periplasm **or growth medium**".

Applicants submit that absent a teaching to screen for very high levels of secreted nuclease, one would not be motivated to combine the references, as applicants have done, with an expectation of success, as the prior art teaches away from the claimed invention by teaching that one must add exogenous nuclease. This argument is not found persuasive, because as stated above and previously, one of ordinary skill in the art would have been motivated to genetically engineer a bacterial strain to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of a fermentation process for the synthesis of industrially important molecules. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and

Bacillus subtilis and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium.

Applicants comments regarding their further interpretation of "an effective amount" as this pertains to commercial scale fermentation processed are acknowledged however applicants are reminded that specific limitations that might be associated with such processes are not currently limitations of the rejected claims, and applicants arguments supporting such limitations that are read into the claims are not persuasive in overcoming the currently rejected claims with the current limitations.

Applicants comments as directed to selection of the disclosed fermenting bacterial strains based upon a screening process are also acknowledged, however Applicants are reminded that the current rejection is based upon the obviousness of the claimed bacterial strains, and the referred to screening processes are not considered relevant to the current rejection as discussed above.

Applicants comments that with respect to claims 2, 4, 3, 5 and 7 that there is no teaching in any reference, singly or in combination, of a fermenting bacterial strain that is capable of growing to densities of at least 50g/l (claim 2), of an aqueous process to manufacture poly(3-hydroxyalkanoates) granule suspension(claim 4), production of polyhydroxyalkanoate to a level of at least 40% of its dry weight (claim 3), of a process for making polysaccharides (claim 5) or of the host strains selected from the group

consisting of *Ralstonia eutropha, Methylobacterium organophilum, Methylobacterium extorquens, Aeromonas caviae, Azotobacter, vinelandii, Alcaligenes latus, Pseudomonas oleovorans, Pseudomonas fluorescens, Pseudomonas putidas, Pseudomonas aeruginosa, Pseudomonas acidophila, Pseudomonas resinovorans, Escherchia coli, and Klebsiella* (claim 7) are acknowledged. Applicants are reminded that as stated above, one of ordinary skill in the art would have been motivated to genetically engineer a bacterial strain to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of a fermentation process for the synthesis of industrially important molecules. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. One of ordinary skill in the art one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. and Atkinson et al. in order to more efficiently produce the desired product, consisting of antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates or polysaccharides. Optimization of fermentation conditions includes the choice of the bacterial host which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that *Alcaligenes eutrophus* has been studied in detail due to its ability to accumulate large amounts of P(3HB) (poly(3-hydroxyalkanoates) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80%

wt/wt of dry cell mass, page 30 through 32). It would have been obvious to use a bacterial strain which grows to a high cell density and/or which produces a high level of the desired product.

Further, Applicants are reminded that claims 4 and 5 are drawn to the bacterial strains of claims 2 and 1, respectively, for use in an aqueous process to manufacture poly(3-hydroxyalkanoates) granule suspension, or for use in a process for making a selected polysaccharide, and as discussed above these uses do not further limit the claimed bacterial strain.

As discussed above, an amount effective to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours is a somewhat broad limitation as a single cleavage event of a nucleic acid molecule could be considered to be encompassed by "degrade"., and thus the bacterial strain taught by Liebl et al. secretes the expressed nuclease in an amount effective to degrade at least 95% of all nucleic acid released following lysis of the cells in less then 24 hours and in the event that applicants disagree that the bacterial strain taught by Liebl et al. produces such a level of nuclease activity, as pointed out by applicants above, one of ordinary skill in the art of protein/product purification from microorganisms will realize that an effective amount does not correlate with just "any amount of nuclease excreted into the medium", and that one would want to degrade as much nucleic acid in the medium as possible which would equate to 100% of the nucleic acid in the medium. As applicants have submitted that genetic engineering of bacteria has been practiced for decades and that commercial fermentation of bacteria to produce amino acids and other products, as

well as the mutagenesis of bacteria and screening for a particular characteristic such as antibiotic resistance, or an increase in the production and secretion of a nuclease, would seem to support the rejection that determination of and engineering of "an effective amount of nuclease to degrade nucleic acid so that recovery of the product is enhanced" is desirable and well within the ability of the ordinary artisan and there is a reasonable expectation of success that one could achieve such an "effective amount to degrade at least 95% of the nucleic acid following lysis of the cells in less than 24 hours.

Further with respect to Applicants comments that there is no teaching of enhancing nuclease activity via gene expression from a modified homologous nuclease gene, as discussed above such would be a reasonable alternative to the introduction of a heterologous gene into a bacterial strain, and as originally stated above by both the examiner and applicant, the level of knowledge of the ordinary artisan in the art of protein/product purification from microorganisms is high, and the genetic engineering of bacteria has been practiced for decades and the commercial fermentation of bacteria to produce amino acids and other products, as well as the mutagenesis of bacteria and screening for a particular characteristic such as antibiotic resistance, or an increase in the production and secretion of a nuclease is a routine practice. Therefore, such a genetic modification of a homologous gene would be desirable so as not to alter a bacterial strain by the introduction of new genes which may have unforeseen deleterious effects on product production, and the reasonable expectation of success high.

Finally as many of applicant's arguments, are made against the references individually, Applicant is reminded that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G. Hutson whose telephone number is 571-272-0930. The examiner can normally be reached on M-F, 7:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Rgh
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